



# Benchtopics in Gene Expression

*A gene expression quarterly*

Introducing Benchtopics, an application-oriented newsletter designed to expand your research capabilities. In each issue, you'll find tips, tools, and techniques specific to your area of interest.

*A forum for  
research  
applications*

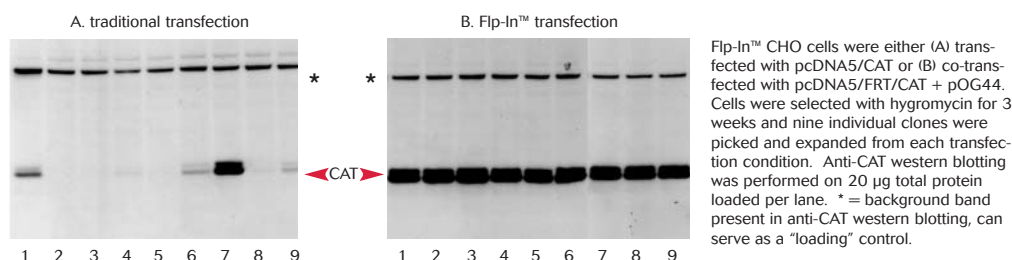
## Flp-In™: Fast, stable mammalian gene expression

The Flp-In™ System for efficient targeted integration saves time when generating stable cell lines by eliminating the need to isolate single clones. The Flp-In™ System integrates a single copy of your gene of interest at a particular genomic locus in every transfected cell. The time-consuming process of isolation and characterization of single cell clones is no longer necessary, as a single Flp-In™ transfection will yield an isogenic, homoge-

nous population of stably expressing cells (Figure 1). In addition, integration of a gene of interest into a single site limits chromosomal position effects on gene expression. This allows for reproducible analysis of multiple genes (or variants of a single gene) without the variability introduced by multiple integrated copies or single integration events at different chromosomal locations. Whether you

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**Figure 1** - Comparison of individual clones generated by Flp-In™ vs. traditional stable transfection



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## ViraPower™ Lentiviral Expression System: Reproducible, stable gene expression in any mammalian cell line

The ViraPower™ Lentiviral Expression System is designed for high levels of stable gene expression in mammalian cells. With this system, you can reproducibly deliver your gene to a full population of dividing or non-dividing cells. Using Lentivirus broadens your ability to analyze gene expression beyond what can be done with adenoviral or other retroviral expression systems. Standard transfection or viral transduction experiments fail to achieve the levels of gene expression needed for experimentally valid results in

some mammalian cell types. These previously difficult gene expression experiments can be easily accomplished using the ViraPower™ Lentiviral Expression System. Whether you're using a hard-to-transfect mammalian cell line, an animal model, or simply want efficient gene delivery, ViraPower™ Lentiviral Expression System offers the significant advantages of stable gene expression and reproducible delivery to dividing and non-dividing cells.

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# Flp-In™ System *continued from cover*

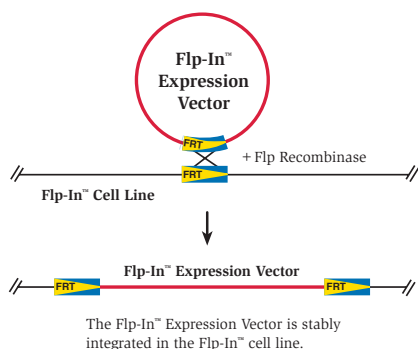
need to generate multiple stable cell lines faster or want to eliminate expression variability in your experiments, Flp-In™ will help you get the results you need.

## How to Flp-In™ a gene

The Flp-In™ System consists of three plasmids: the target site vector (pFRT/*lacZeo* or pFRT/*lacZeo2*), an expression vector (pEF5/FRT/V5-TOPO®, pcDNA5/FRT/V5-His™, or pSecTag/FRT/V5-His-TOPO®), and the vector that transiently expresses Flp recombinase (pOG44). In a straightforward two-step process, you can insert your gene of interest into the same genetic locus in every cell (Figure 2). The first step in generating isogenic expressing cells is to create a Flp-In™ Cell Line using the target site vector to integrate a single FRT site in the host cell genome. Or you can save time and effort by using one of several premade, tested Flp-In™ Cell Lines available in many common cell types (Table 1). Once you have a Flp-In™ Cell Line, perform a simple co-transfection with the Flp-In™ Expression Vector containing the gene of interest and a source of Flp recombinase (the pOG44 vector). The result is a Flp recombinase-mediated integration of the expression vector at a single, specific genomic site in every transfected cell. Since every transfected cell is the same, you can

**Figure 2** - Stable integration using Flp-In™

A Flp-In™ expression vector containing a FRT site and the gene of interest is transfected into the Flp-In™ Cell Line. In the presence of Flp Recombinase, this vector will specifically integrate at the genomic FRT site.



quickly select your stable cells as a population rather than isolating single clones.

## Stable expression in more cell types

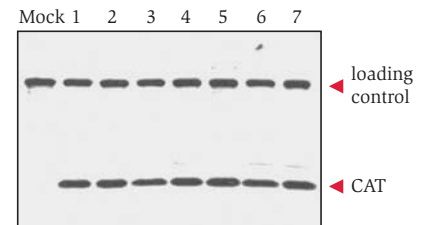
Flp-In™ expression vectors are available with either the CMV promoter or EF-1α promoter to optimize expression in different cell types. CMV is a proven and popular promoter for expression in a broad range of cell lines. However, in some cell lines, CMV promoter activity is low or can be down-regulated, making the cellular EF-1α in the pEF6/FRT/V5-TOPO® or pEF6/V5-His-DEST™ vector a better choice. The EF-1α promoter is derived from the human elongation factor-1α subunit gene and is known to have strong, constitutive expression in most mammalian cell types. Now you can get high level expression in Flp-In™ Cell Lines where stable CMV expression is absent or inconsistent—such as non-transformed primary tissue or lymphoid cells.

## Efficiently expressing cell lines

Seven Flp-In™ Cell Lines are currently available (Table 1). Each has been tested by Southern blot to have a single integration site and transfected for functional expression studies. Integration occurs at a single site, eliminating clonal variability. You'll achieve similar levels of protein expression from each clone (Figure 3). For two of these cell lines, baby hamster kidney (BHK) cells and a mouse fibroblast cell line (NIH-3T3), the pEF/FRT/V5-TOPO® or pEF/FRT/V5-His-DEST™ vector gives superior results (Figure 4). In these cell lines, the CMV promoter appears to be down-regulated over time, whereas the EF-1α promoter consistently

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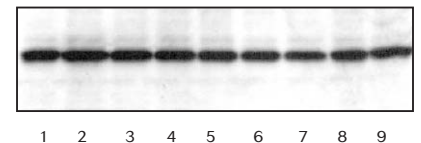
**Figure 3** - Stable CAT expression from Flp-In™ clones



Flp-In™-Jurkat cells were co-transfected with pEF5/FRT/CAT + pOG44. Hygromycin B-resistant foci were picked, expanded, and analyzed for CAT expression by western blot using an anti-CAT antibody.

Mock: Flp-In™-Jurkat cells alone Lanes 1-7: CAT expression from individual Hygromycin B-resistant clones

**Figure 4** - Stable expression of CAT in Flp-In™ 3T3 cells



Flp-In™ 3T3 cells were transfected with pEF5/FRT/V5™-CAT and pOG44 at a 9:1 ratio. Stable cells were selected for two weeks in Hygromycin and nine individual clones were expanded in separate cultures and analyzed by anti-CAT western blotting.

**Table 1** - Flp-In™ Cell Lines

Flp-In™ Cell Line	Source	Quantity	Cat. No.
Flp-In™ 293	Human embryonic kidney	3x10 <sup>6</sup> cells	R750-07
Flp-In™ CV-1	Green monkey kidney	3x10 <sup>6</sup> cells	R752-07
Flp-In™ CHO	Chinese hamster ovary	3x10 <sup>6</sup> cells	R758-07
Flp-In™ BHK <b>NEW!</b>	Baby hamster kidney	3x10 <sup>6</sup> cells	R760-07
Flp-In™ 3T3 <b>NEW!</b>	Mouse fibroblast	3x10 <sup>6</sup> cells	R761-07
Flp-In™ Jurkat <b>NEW!</b>	Acute T-cell leukemia	3x10 <sup>6</sup> cells	R762-07

# ViraPower™ Lentiviral Expression System *continued from cover*

## How ViraPower™ Lentiviral Expression works

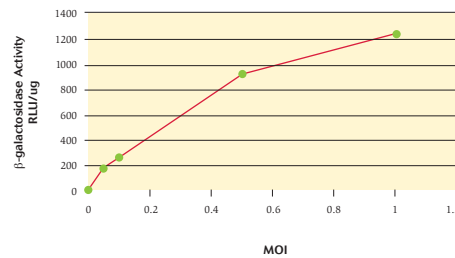
Follow a few basic steps to get high-level stable expression using the ViraPower™ Lentiviral Expression System (Figure 1). Clone your gene of interest into a pLenti6/V5 Directional TOPO® or Gateway™ vector. These vectors have a CMV promoter for high-level expression and C-terminal V5 tag for convenient detection with an Anti-V5 antibody. They also carry the Blasticidin resistance gene to allow rapid selection of transduced cells. Complete a standard co-transfection with the pLenti6/V5™ vector containing your gene of interest and the ViraPower™ Packaging Mix that provides helper functions and viral packaging proteins *in trans*. A replication-incompetent packaged viral particle that contains your gene of interest will be generated and extruded into the supernatant. These viral particles can transduce cells only once and will not produce infectious progeny. Following harvest and titering of the viral supernatant, simply add the viral particles to any cultured mammalian or primary cell line to generate a stable, Blasticidin-resistant cell line with high-level protein expression from the CMV promoter.

## Control Expression

Unique to viral transduction is the ability to reproducibly modulate results by transducing at a selected number of virus per cell. A multiplicity of infection (MOI) of 1 is

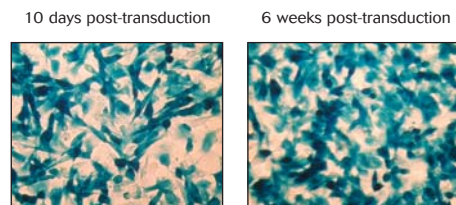
defined as the number of viral particles added to your cells that equals the number of cells in your culture. Theoretically one viral particle transduces one cell.

**Figure 2** - Gene expression is directly correlated with multiplicity of infection (MOI)



HT1080 cells were transduced in duplicate with pLenti6/V5-GW/lacZ™ virus at multiplicities of infection (MOI) of 0.05, 0.1, 0.5 and 1. Forty-eight hours later, cells were harvested and analyzed for β-galactosidase activity.

**Figure 3** - Long-term gene expression from lentiviral vector delivery



HT1080 cells were transduced with pLenti6/V5-GW/lacZ™ lentiviral vector and stably selected with 10 μg/ml Blasticidin. Cultures were stained with β-galactosidase 10 days and 6 weeks post-transduction.

ViraPower™ Lentiviral System allows you alter MOI to optimize gene delivery to a population of cells. To demonstrate the relationship between expression and MOI, HT1080 human fibrosarcoma cells were transduced at various MOIs ranging from 0.05 to 1. As shown in Figure 2, the level of gene expression correlates with MOI. This precise control to transduce at a specific MOI effectively lets you regulate the number of integrated gene copies per cell. Regardless of what type of cells you work with, you'll get efficient and reproducible transient expression detectable within 24 hours. Expression remains high, and you can easily select using Blasticidin for stable expression clones (Figure 3).

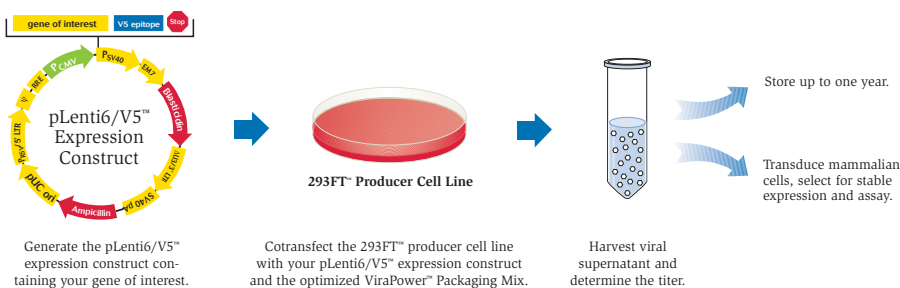
## Non-dividing cells are an option

Traditional Moloney (MLV)-based retroviral systems require a round of host cell division in order to enter the nucleus and integrate into the host genome. With ViraPower™ Lentiviral Expression System, your gene is actively imported into the nucleus so non-dividing cells are readily transduced. Following transduction in non-dividing cells, your gene of interest will become stably integrated into the host cell genome with very high efficiency. This significantly expands your potential transducible targets to include growth- or drug-arrested cells, non-dividing primary cultures, neuronal cells, and animal tissues.

To demonstrate the transduction of non-dividing cells, we compared the ability of a traditional retroviral and the ViraPower™ Lentiviral System to express in growth arrested cells. Actively growing cultured cells can be arrested at specific phases of the cell cycle using a variety of drugs. One commonly used drug is aphidicolin, which reversibly binds to DNA polymerase delta and arrests cells at the G1/S transition. Either a standard retroviral vector or the pLenti6/V5™ Gateway™ control vector carrying a lacZ gene was transduced at an MOI of 1 into HT1080 cells that were either

*continued on back cover*

**Figure 1** - How ViraPower™ Lentiviral Expression System works



# The *Pichia* Expression System: Maximize your protein yields

The *Pichia* Expression System has become a well-recognized system for high-level production of recombinant protein. Since the system has become commercially available, numerous researchers have chosen *Pichia* as their expression host of choice. It's the only system that offers the benefits of *E. coli* (high-level expression, easy scale-up, and inexpensive growth) combined with the advantages of expression in a eukaryotic system (protein processing, folding, and posttranslational modifications). This makes the *Pichia* Expression System an ideal choice for both laboratory research as well as industrial applications.

## How *Pichia* expression works

*Pichia pastoris* is a methylotrophic yeast that can utilize methanol as a carbon source in the absence of a repressing carbon source. When methanol is the only carbon source available, the alcohol oxidase promoter (AOX1) is induced. This promoter is extremely strong because *Pichia pastoris* generates large amounts of this enzyme. Typically > 30% of the total soluble protein in methanol-induced cells is alcohol oxidase (1). The AOX1 promoter has been characterized and incorporated into a number of *Pichia* vectors to drive high-level, tightly controlled expression of the gene of interest.

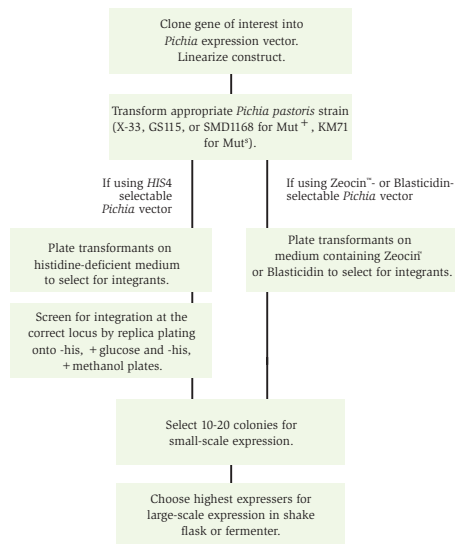
For high-level expression that isn't dependent on methanol for induction, vectors are available that use the constitutive promoter from the glyceraldehyde-3-phosphate dehydrogenase (GAP) gene. Both inducible and constitutive expression constructs integrate into the *Pichia pastoris* genome, creating a stable host that generates extremely high expression levels.

## Easy-to-use system

The *Pichia* Expression System has been engineered to make it as easy to use as a bacterial system (Figure 1). It offers the following advantages:

- A simple, convenient method for preparing and transforming competent *Pichia*

**Figure 1** - Flowchart of expression



*pastoris* cells, eliminating the time-consuming preparation of spheroplasts

- A number of vectors containing the Zeocin™ or Blasticidin resistance gene to allow direct selection of transformed cells without preparation of drop-out medium or screening through background colonies
- A growth medium that doesn't require growth factors or supplements, which are costly and make protein purification more difficult

Additionally, these vectors contain fusion tags that simplify the purification and analysis of expressed proteins.

## Easy scale-up

*Pichia pastoris* is easily adaptable to large-scale fermentation, making the system ideal for large-scale production of functional protein. Fermenters as large as 10,000 liters

**Table 1** - Examples of heterologous proteins expressed in *Pichia pastoris*

Protein expressed	Expression (mg/L)	Reference
<b>Bacterial proteins</b>		
Tetanus toxin fragment C	12,000	Clare, J.J. <i>et al.</i> (1991) <i>Bio/Technology</i> <b>9</b> : 455–460
$\alpha$ -amylase	2,500	Paifer, E. <i>et al.</i> (1994) <i>Yeast</i> <b>10</b> : 1415–1419
T2A peroxidase	2,470	Thomas, L. <i>et al.</i> (1998) <i>Can. J. Microbiol.</i> <b>44</b> : 364–372
<i>C. botulinum</i> neurotoxin fragment	78	Smith, L.A. (1998) <i>Toxicon</i> <b>36</b> : 1539–1548
<b>Yeast proteins</b>		
Catalase L	2,300	Calera, J.A. <i>et al.</i> (1997) <i>Infect. Immun.</i> <b>65</b> : 4718–4724
Glucoamylase	400	Fierobe, H.-P. <i>et al.</i> (1997) <i>Protein Expr. Purif.</i> <b>9</b> : 159–170
Lipase	60	Minning, S. <i>et al.</i> (1998) <i>J. Biotechnol.</i> <b>66</b> : 147–156
<b>Plant proteins</b>		
Hydroxynitrile lyase	22,000	Hasslacher, M. <i>et al.</i> (1997) <i>Protein Expr. Purif.</i> <b>11</b> : 61–71
Wheat lipid transfer protein	720	Klein, C. <i>et al.</i> (1998) <i>Protein Expr. Purif.</i> <b>13</b> : 73–82
Aeroallergen	60	Huecas, S. <i>et al.</i> (1999) <i>Eur. J. Biochem.</i> <b>261</b> : 539–546.
<b>Invertebrate proteins</b>		
Hirudin	1,500	Rosenfeld, S.A. <i>et al.</i> (1996) <i>Protein Expr. Purif.</i> <b>8</b> : 476–482.
Spider dragline silk protein	663	Fahnestock, S.R. <i>et al.</i> (1997) <i>Appl. Micro. Biotechnol.</i> <b>47</b> : 33–39
Honeybee olfactory protein	200	Danty, E. <i>et al.</i> (1999) <i>J. Neuroscience</i> <b>19</b> : 7468–7475
<b>Mammalian proteins</b>		
Mouse gelatin	14,800	Werten, M.W. <i>et al.</i> (1999) <i>Yeast</i> <b>15</b> : 1087–1096
Porcine carboxypeptidase B	200	Ventura, S. <i>et al.</i> (1999) <i>J. Biol. Chem.</i> <b>274</b> : 19925–33
Human tumor necrosis factor	10,000	Sreekrishna, K. <i>et al.</i> (1989) <i>Biochemistry</i> <b>28</b> : 4117–4125
Human IGF-1	600	Brierley, R.A. (1998) <i>Methods Mol. Biol.</i> <b>103</b> : 149–177
Human CD38	455	Munshi, C.B. (1997) <i>Methods Enzymol.</i> <b>280</b> : 318–330
15N-Interferon $\tau$	10	Johnson, T.M. <i>et al.</i> (1999) <i>J. Interferon Cytokine Res.</i> <b>19</b> : 631–636

have been used to produce recombinant proteins in *Pichia*. This results in more efficient protein production and, ultimately, significant cost savings.

### Proven expression

A wide variety of proteins have been expressed using the *Pichia* Expression System, including enzymes, proteases, protease inhibitors, receptors, single-chain antibodies, and regulatory proteins. Some proteins have been expressed to levels as high as grams per liter (Table 1). The rapidly growing citation list reflects the utility and reliability of this system.

### The power of *Pichia*

Maximize your expression. Choose the *Pichia* Expression System for high-level, easy-to-use, proven expression. Three *Pichia* kits are available with everything you need to get started. The kits include expression vectors, *Pichia* strains, a transformation kit

and primers (Table 2). For more information on the *Pichia* Expression System and our wide variety of expression vectors, visit our web site ([www.invitrogen.com/pichia](http://www.invitrogen.com/pichia)).

### References:

1. Cregg, J.M. *et al.* (1993) *Bio/Technology* 11: 905-910.

Product	Quantity	Cat. no.
EasySelect™ <i>Pichia</i> Expression Kit	1 kit	K1740-01
Original <i>Pichia</i> Expression Kit	1 kit	K1710-01
Multi-Copy <i>Pichia</i> Expression Kit	1 kit	K1750-01

**Table 2** – *Pichia* expression kit components

	EasySelect™ <i>Pichia</i> Kit	Multi-Copy <i>Pichia</i> Kit	Original <i>Pichia</i> Expression Kit
Vectors	pPICZ A, B, & C (20 µg each) pPICZα A, B, & C (20 µg each)	pPIC9K (20 µg) pPIC35K (20 µg) pAO815 (20 µg)	pPIC9 (10 µg) pPIC3.5 (10 µg) pHIL-D2 (10 µg) pHIL-S1 (10 µg)
Strains	X-33 (Mut <sup>+</sup> , His <sup>+</sup> ) GS115 (Mut <sup>+</sup> ) KM71 (Mut <sup>s</sup> ) GS115/pPICZ/ <i>lacZ</i> (control)	GS115 (Mut <sup>+</sup> ) KM71 (Mut <sup>s</sup> ) GS115/β-gal (control) GS115/albumin (control)	GS115 (Mut <sup>+</sup> ) KM71 (Mut <sup>s</sup> ) GS115/β-gal (control) GS115/albumin (control)
Transformation Reagents	<i>Pichia</i> EasyComp™ Kit (120 transformations)	Spheroplast Module (50 transformation)	Spheroplast Module (50 transformations)
Sequencing Primers	5' AOX1 3' AOX1 α-factor	5' AOX1 3' AOX1 α-factor	5' AOX1 3' AOX1 α-factor
Media and Supplements	YP base medium (2 pouches)* YP base agar (2 pouches)*  Yeast Nitrogen Base (1 pouch)** Zeocin™ (250 mg)	YP base medium (2 pouches)* YP base agar medium (2 pouches)* Yeast Nitrogen Base medium (1 pouch)**	YP base medium (2 pouches)* YP base agar medium (2 pouches)* Yeast Nitrogen Base medium (1 pouch)**

\* Each pouch contains reagents to prepare 1 liter of medium.

\*\* Each pouch contains reagents to prepare 500 ml of 10X YNB.

## Flp-In™ System continued from page 2

resulted in long-term stable expression. So if you are creating stable cell lines, the Flp-In™ System can save you time and eliminate variability in your experiments. Call and order today. For more information on the Flp-In™ System and other Flp-In™ components, please visit [www.invitrogen.com](http://www.invitrogen.com).

Product	Quantity	Cat. no.
pEF5/FRT/V5-DEST Gateway™ Vector Set	6 µg	V6020-20
pEF5/FRT/V5 Directional TOPO® Expression Kit	1 kit	K6035-01
Flp-In™ -BHK Cell Line	3 x 10 <sup>6</sup> cells	R760-07
Flp-In™ -3T3 Cell Line	3 x 10 <sup>6</sup> cells	R761-07
Flp-In™ pcDNA5/FRT Complete System	1 kit	K6010-01

# pET Directional TOPO®: Clone today, express tomorrow

pET Directional TOPO® Expression Vectors are designed to yield high levels of protein and streamline cloning. Researchers who want high-level inducible expression from a T7 promoter in *E. coli* rely on pET vectors as their number-one choice. Directional TOPO® Cloning allows you to clone constructs in a 5-minute benchtop reaction with high reliability and less clone screening time.

## How Directional TOPO® works

The pET Directional TOPO® Expression kits enable unidirectional cloning of blunt-end PCR products generated with proofreading enzymes such as Platinum® *Pfx* DNA Polymerase. The mechanism for Directional TOPO® Cloning is simple (Figure 1).

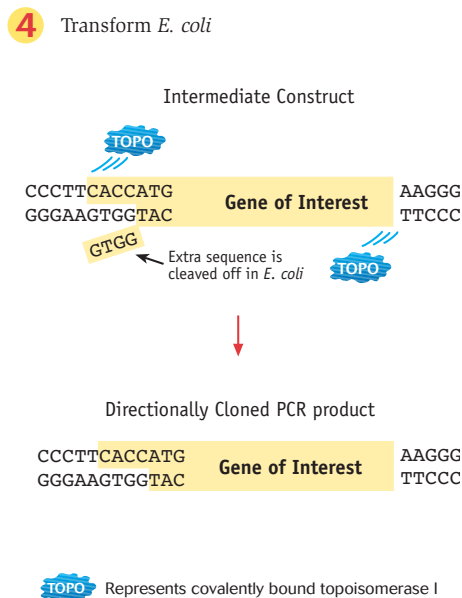
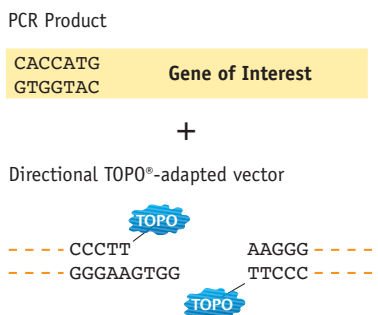
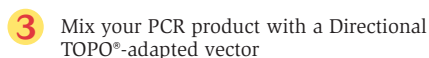
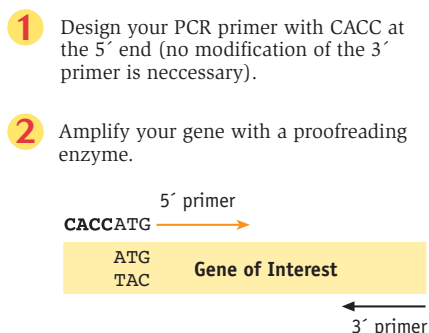
Design the 5' PCR primer with CACC at the 5' end. No modification of the 3' primer is necessary. Then amplify your gene as usual with a proofreading PCR enzyme. Once the PCR product and the vector are mixed, a four-base overhang on the TOPO®-adapted vector hybridizes to the complementary sequence in your PCR product via strand invasion. Topoisomerase I then rapidly ligates the PCR product in this orientation. Following transformation into *E. coli*, >90% of recombinants will contain a directionally cloned PCR product ready for expression.

## Directional pET vectors

When cloned into a pET vector, your gene of interest is placed under the control of the exceptionally strong T7 promoter. Expression from this promoter is controlled by the availability of T7 RNA polymerase within the host cell. This regulation is accomplished by using a BL21 *E. coli* host that contains a chromosomal copy of T7 RNA polymerase under the control of an inducible promoter.

With pET Directional TOPO® Expression Kits, expression of recombinant protein is initiated by addition of the inducer IPTG to the culture medium. Upon induction, T7

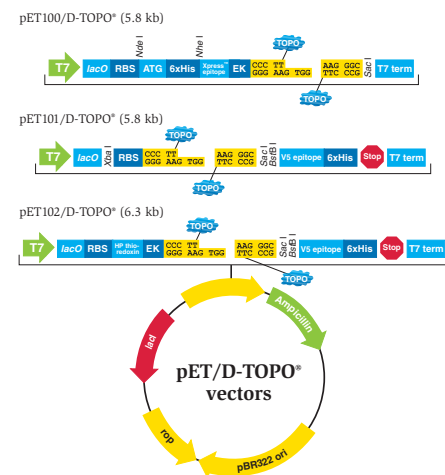
**Figure 1** - How Directional TOPO® works



RNA polymerase is produced, initiating transcription from T7 promoter and driving high-level expression of your gene of interest. The additional *lacO* element in front of the T7 promoter (Figure 2) further reduces basal expression by binding *lacI* which is displaced in the presence of IPTG.

Further refinements included in the pET Directional TOPO® Expression Vectors include features that allow for protein detection and purification (Figure 2 and Table 1). You can choose from three pET Directional TOPO® expression vectors: pET100/D-TOPO®, pET101/D-TOPO® and pET102/D-TOPO®. They differ from each other by the presence and location of purification, detection, and solubility tags, such as Xpress™, 6xHis, V5 and thioredoxin (Table 1, next page).

**Figure 2** - pET Directional TOPO® Expression Vectors



## Tags

**Xpress™**: Asp-Leu-Tyr-Asp-Asp-Asp-Lys-

**V5**: Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr

**6xHis**: His-His-His-His-His

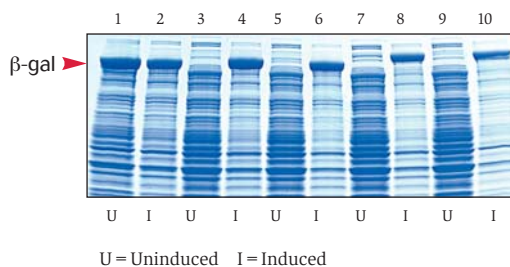
**Thioredoxin**: 11.7 kDa protein known to increase solubility and translational efficiency

Monoclonal antibodies are available to the above tags for detection by western blot or ELISA.

### Proven expression

pET Directional TOPO® Expression Vectors give you the same high-level expression that you would find with original pET vectors. To demonstrate, expression of  $\beta$ -galactosidase was compared in pET Directional TOPO® vectors and their non-TOPO®-adapted counterparts (Figure 3).

**Figure 3** - Expression of  $\beta$ -galactosidase using pET Directional TOPO® expression vectors



The *lacZ* gene was Directionally TOPO® Cloned into pET100/D-TOPO®, pET101/D-TOPO® and, pET102/D-TOPO® and cloned using the restriction digest method into the non-TOPO®-adapted pET15b and pET32a vectors. Constructs were transformed into BL21 Star™(DE3) *E. coli*. A single transformant from each transformation was used to inoculate 1 ml LB medium supplemented with 100  $\mu$ g/ml ampicillin. Induction with 1mM IPTG was performed at  $OD_{600}$  = 0.5. Two and one-half hours postinduction, cultures were harvested by centrifugation. Pellets were resuspended in 300  $\mu$ l sample buffer. Ten microliters of each sample was analyzed on a 4-20% Novex® Tris-Glycine gel.

**Note:** pET15b contains an N-terminal 6xHis tag while pET32a contains an N-terminal thioredoxin fusion and a C-terminal 6xHis tag.

Lanes 1 and 2: pET15b/*lacZ*  
 Lanes 3 and 4: pET101/D-TOPO®/*lacZ*  
 Lanes 5 and 6: pET100/D-TOPO®/*lacZ*  
 Lanes 7 and 8: pET102/D-TOPO®/*lacZ*  
 Lanes 9 and 10: pET32a/*lacZ*

### Complete kit

With each pET Directional TOPO® Expression kit you will get everything you need to ensure successful and rapid cloning and high-level protein expression in *E. coli* (Table 2). In addition to topoisomerase I-activated directional pET vector, PCR reagents, expression controls, cloning and expression competent cells are also included. One Shot® TOP10 is a highly efficient (> 1x10<sup>9</sup>), versatile cloning strain which is suitable for most cloning applications. One

Shot® TOP10 should be used for high efficiency cloning of your PCR product into the pET vector.

One Shot® BL21 Star™ is included in the kit as an expression strain that has been engineered to boost protein expression levels by enhancing mRNA stability so you can achieve higher yields. For more information on pET Directional TOPO® Expression kits, call Invitrogen today or visit [www.invitrogen.com](http://www.invitrogen.com).

Product	Reactions	Cat. no.
pET 100 Directional TOPO® Expression Kit	20	K100-01
pET 101 Directional TOPO® Expression Kit	20	K101-01
pET 102 Directional TOPO® Expression Kit	20	K102-01

**Table 1** - Features of pET Directional TOPO® expression vectors

Vector	Size	Purification tag	Detection tag	Solubility tag
pET100/D-TOPO®	5.8 kb	N-terminal 6xHis	N-terminal Xpress™	NA
pET101/D-TOPO®	5.8 kb	C-terminal 6xHis	C-terminal V5	NA
pET102/D-TOPO®	6.3 kb	C-terminal 6xHis	N-terminal Xpress™ C-terminal V5	N-terminal thioredoxin

**Table 2** - Contents of the pET Directional TOPO® expression kits

Expression vector	pET100/D-TOPO®, pET101/D-TOPO®, or pET102/D-TOPO®	
Expression control vector	pET100/D/ <i>lacZ</i> , pET101/D/ <i>lacZ</i> , or pET102/D/ <i>lacZ</i>	
PCR Reagents	dNTP mix Salt solution Sterile water	Sequencing primers PCR controls
Competent <i>E. coli</i>	One Shot® TOP10 Chemically Competent <i>E. coli</i> (21 x 50 $\mu$ l) BL21 Star™(DE3) One Shot® Competent Cells (21 x 50 $\mu$ l)	

# ViraPower™ Lentiviral Expression System *continued from page 3*

actively growing or growth arrested by the addition of aphidicolin (Figure 4A). Actively growing cells expressed  $\beta$ -galactosidase from both vectors; however, only pLenti6/V5-GW/*lacZ*™ expressed in the non-dividing cells.

In addition, pLenti6/V5-GW/*lacZ*™ was tested for ability to transduce primary fibroblasts, which are strongly contact inhibited and can be maintained for many weeks arrested in a G0 quiescent state when maintained as a confluent culture. Similar to the

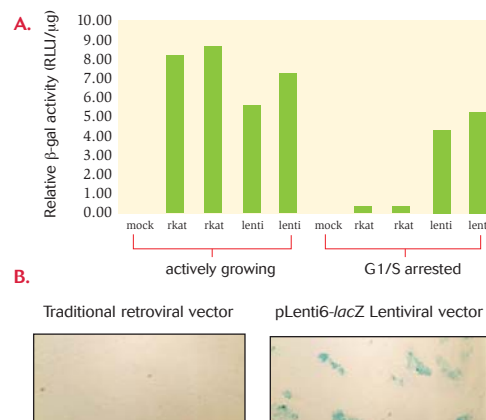
results in the aphidicolin-arrested cells, the non-dividing quiescent fibroblasts could only be transduced by the pLenti6/V5™ lentiviral (Figure 4B). In this case, at an MOI of 1, about 50% of the quiescent primary cells were transduced.

## Get results with ViraPower™

Get powerful expression in previously hard-to-use cell types and get the results you've been waiting for. Call and order your ViraPower™ Lentiviral Expression Kit today.

Product	Quantity	Cat. no.
ViraPower™ Lentiviral Directional TOPO® Expression Kit (pLenti6/V5 Directional TOPO® Cloning Kit, ViraPower™ Support Kit, and Cells)	1 kit	K4950-00
ViraPower™ Lentiviral Gateway™ Expression Kit (pLenti6/V5-DEST™ Gateway™ Vector Pack, ViraPower™ Support Kit, and Cells)	1 kit	K4960-00
pLenti6/V5 Directional TOPO® Cloning Kit	1 kit	K4955-10
pLenti6/V5-DEST™ Gateway™ Vector Pack	6 $\mu$ g	V496-10
ViraPower™ Lentiviral Support Kit (ViraPower™ Packaging Mix, Lipofectamine™ 2000, Blastidicin)	20 rxns	K4970-00
ViraPower™ 293FT™ Cell Line	3 x 10 <sup>6</sup> cells	R700-07

**Figure 4 - Long-term gene expression from lentiviral vector delivery**



**A.** HT1080 cells were either actively growing or growth arrested at G1/S by aphidicolin and transduced at an MOI of 1, in duplicate, with either a traditional retroviral vector (rkat) or pLenti6/V5-GW/*lacZ* lentivirus (lenti). Forty-eight hours post transduction, cell lysates were analyzed for  $\beta$ -galactosidase activity.

**B.** Contact-inhibited non-dividing quiescent primary human foreskin fibroblasts were transduced with retrovirus and lentivirus at an MOI of 1 and  $\beta$ -galactosidase stained 48 hours post transduction.

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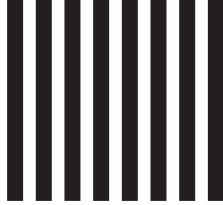
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